

Incidental Finding of *Myxobolus* Spores (Protozoa: Myxozoa) in Stool Samples from Patients with Gastrointestinal Symptoms

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Myxozoan spores were detected in fecal samples from three patients presenting with abdominal pain and/or diarrhea. The spores were identical to those of *Myxobolus plectroplites*, a previously described pathogen from the freshwater fish *Plectroplites ambiguus*. All patients had recently eaten fish caught from local waters, and frozen fillets of such fish were found to be infected with *M. plectroplites* cysts. The passage of spores unchanged through the alimentary tract suggests they were incidental findings unrelated to clinical symptoms, especially since other enteric pathogens were present in two patients.

Many infectious microorganisms may cause gastroenteritis in humans, including viruses, bacteria and protozoan and helminth parasites. The laboratory examination of stool samples from diarrheic patients is frequently used to facilitate diagnosis, especially for parasitic infections, although the identification of organisms can be complicated by artifacts, contaminants, pseudoparasites, or fecal debris. Infections with enteric protozoa (flagellates, amoebae, coccidia, and ciliates) are conventionally diagnosed by the demonstration of motile or encysted organisms in fecal preparations (3). Developmental stages of other protozoa, especially spores of microsporidian species, have also been encountered as endoparasites, mainly in immunocompromised patients (1) or as incidental findings (7). More recently, spores of a myxosporean parasite (*Heneguya salminicola*) were detected for the first time in fecal samples from two human patients (6), in one case being mistaken for human spermatozoa and leading to a suspicion of sexual abuse. The spores were passed undigested in feces apparently after the consumption of infected salmon, and they were not thought to have contributed to any clinical symptoms. In this paper, we report on a further three cases in which myxosporean spores were detected in fecal samples from patients presenting with gastrointestinal symptoms in southeast Queensland, Australia.

Case 1. A 44-year-old male presented with recurrent episodic abdominal pain. Standard hematological and multiple biochemical assessments did not reveal any specific abnormalities. Symptoms persisted for 6 weeks, and a stool sample was submitted for laboratory examination. Light microscopy revealed the presence of small numbers of ovoid spores (~11 by 7 μm) in wet preparations (Fig. 1a), formalin-ethyl acetate concentrates (Fekal-Contrate kit), and smears stained with modified acid-fast (Fig. 1b) and modified trichrome (Fig. 1c) stains (3). The spores did not stain with calcofluor white chemofluorescent stain, and they were not autofluorescent (3). No other parasites were detected, and no bacterial pathogens were isolated. Hemoglobin levels had dropped from 144 to 129 g/liter, and a mild leukocytosis (10,600/ μl) with neutrophilia (8,870/ μl) and monocytosis (1,180/ μl) was evident. Symptoms

persisted for another 4 weeks, and then gastric, duodenal, and colonic biopsies were performed. Apart from a mild melanosis coli, there were no abnormalities or pathogens seen. The patient was treated with Flagyl (250 mg three times daily for 7 days) and the symptoms abated. Subsequent blood and fecal samples revealed no abnormalities, parasites, or bacterial pathogens. The patient has remained well, and no underlying medical conditions were noted. At the interview, the patient recalled eating freshwater fish, *Plectroplites ambiguus* (commonly known as golden perch, callop, or yellow belly), prior to the onset of symptoms. The fish were caught in a local creek, filleted, and frozen. The thawed fillets were cooked in an oven but were memorable in that they tasted "awful" and "muddy." His spouse also ate part of the same fish but did not become ill. The patient provided the laboratory with frozen fish from the same batch that he had eaten prior to feeling ill. Histological examination revealed the presence of small numbers of cysts containing identical spores in four of five muscle blocks examined (Fig. 2).

Case 2. A two-year-old female with a history of lactose and sugar intolerance presented with abdominal pain and bloody diarrhea. *Campylobacter jejuni* was isolated from a stool sample, and moderate numbers of ovoid spores (11 by 7 μm) were detected in fecal preparations. Symptoms resolved spontaneously, and no parasites or pathogens were found in subsequent samples. The child had been fed wild-caught golden perch from a local dam three to four times weekly since she was 1 year old. She had eaten fried fish the night before she became ill. Other family members and friends had also eaten fish from the same batch, but they remained asymptomatic. Two residual samples of golden perch were sent to the laboratory, but no myxozoan cysts or spores were detected upon histological examination.

Case 3. A 26-year-old pregnant female presented with abdominal pain, and a stool sample was submitted for examination. Microscopy revealed the presence of leukocytes, occasional vacuolar forms of *Blastocystis hominis*, and small numbers of ovoid spores (11 by 7 μm) in fecal smears and concentrates. The patient was treated with Flagyl and has been well since. No parasites or bacterial pathogens were detected in repeat samples taken 1 week later. The patient and her family had recently caught a large batch of golden perch in a local municipal dam and had frozen fillets to barbecue on weekends. She had dined on these fish twice before becoming ill. On the second

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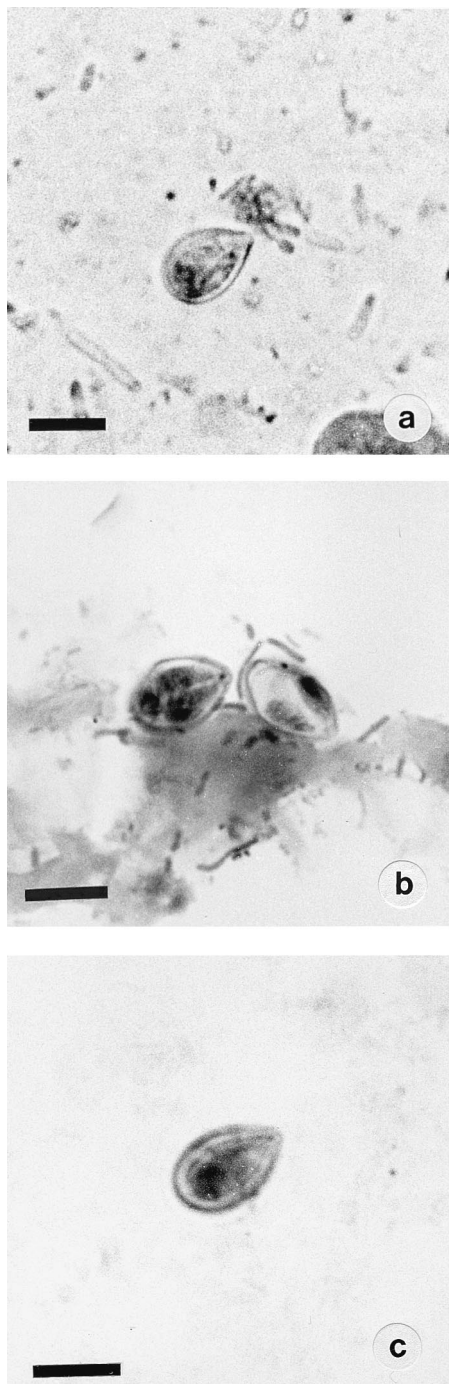


FIG. 1. Light micrographs of mature spores of *M. plectroplites* detected in human fecal preparations. (a) Wet smear with iodine stain (spores evident as dark pyriform bodies); (b) fecal concentrate with modified carbol-fuchsin stain (spore wall and enclosed sporoplasmic and capsulogenic cells stain acid fast); (c) fecal concentrate with modified trichrome stain (spores conspicuous as darkly stained pear-shaped bodies). Bar = 10 μ m.

occasion, she noted that the fish had tasted "slimy" and undercooked and that her guests had not eaten their portions because the fish tasted "funny." She alone became ill. The patient sent the remaining frozen fillets to the laboratory, and histological examination revealed the presence of numerous cysts containing spores in six of seven samples.

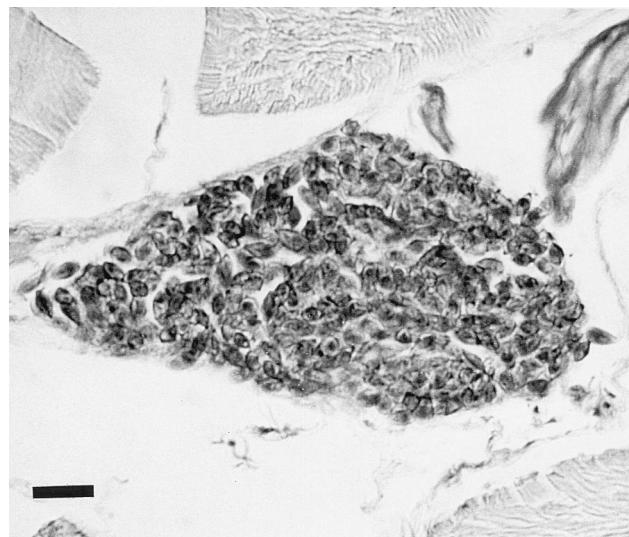


FIG. 2. Light micrograph of *M. plectroplites* cyst detected in connective tissue in the musculature of the freshwater fish *P. ambiguus* (with periodic acid-Schiff staining). Bar = 20 μ m.

Parasite description. The spores detected in fecal samples from the three patients were monomorphic and uniform in size, shape, and appearance. They were ovoid and dorsoventrally flattened, measuring 11 to 12 μ m (mean \pm standard error = 11.7 ± 0.15 μ m; $n = 40$) long, 7.0 to 8.0 μ m (7.8 ± 0.13 μ m; $n = 40$) wide, and 4.0 μ m ($n = 5$) thick. The spores exhibited bilateral symmetry, with two adjacent pyriform polar capsules located at the thinner anterior end and an oval sporoplasm at the rounded posterior end. The polar capsules were equal in size, measuring 5 to 6 μ m (5.6 ± 0.16 μ m; $n = 20$) long by 2 to 3 μ m (2.3 ± 0.15 μ m; $n = 20$) wide. Transmission electron microscopy confirmed that the spores were formed by two valvogenic cells enclosing two capsulogenic cells and a single sporoplasmic cell (Fig. 3). The polar capsules contained coiled polar filaments ranging in width from 90 to 130 nm. Parasitic cysts detected in fish fillets ranged in size from 40 to 350 μ m long by 20 to 150 μ m wide. They were located both

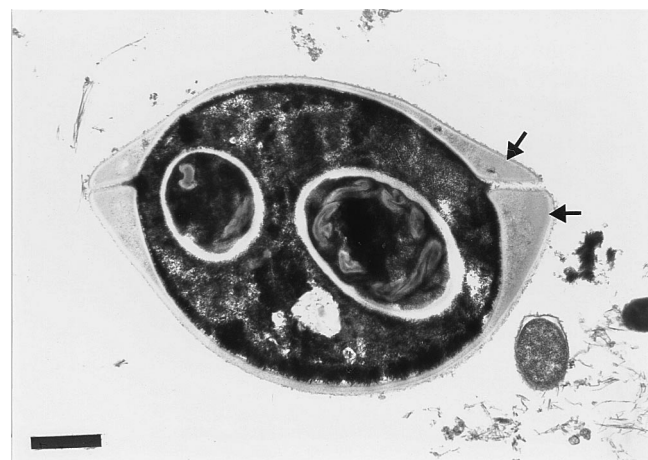


FIG. 3. Transmission electron micrograph of cross-section through mature *M. plectroplites* spore recovered from human fecal sample. Shown are two pale valvogenic cells (arrows) enclosing dark sporoplasm that contains two capsulogenic cells. Bar = 1 μ m.

within skeletal muscle fibers and interfascial connective tissues. They were bounded by thin membranous walls and contained numerous refractile spores which were identical in size, shape, and appearance to those detected in fecal samples. Transmission electron microscopy revealed the spores to have the same ultrastructural characteristics as those from fecal samples, these being consistent with their identification as mature spores of a bivalvulid myxosporean parasite. In particular, the spores conformed with the original description of *Myxobolus plectroplites* found in the connective tissues of *P. ambiguus* in Queensland (4). The original report described ovoid spores measuring 10 to 12 μm by 7 to 8 μm with two anterior polar capsules measuring 5 by 2 μm .

Myxozoa are commonly found as endoparasites in aquatic poikilotherm vertebrates (especially fish) and invertebrates. They comprise an enigmatic group thought variously to belong to the Protozoa, Cnidaria, or Bilateria (8). Irrespective of their phylogenetic origins, they are formally recognized as a separate phylum containing two classes: the Myxosporidia, which infect aquatic vertebrates (especially fish, amphibians, and reptiles), and the Actinosporidia, which infect aquatic invertebrates (especially oligochaetes and sipunculids) (5). Some 800 species have been described, but the life cycles of most are not known. Recent studies have indicated that alternation between actinosporidian stages in oligochaetes and myxosporidian stages in fish may occur, at least for some 13 freshwater species examined so far (5). Most myxosporidian species occur as histozoic parasites in fish, and infections are characterized by the formation of tissue cysts containing distinctive multicellular valved spores enclosing multiple polar capsules. Heavy infections have been associated with gross deformities, tissue lesions, organ malfunction, and postmortem myoliquefaction in a variety of freshwater and marine fish species (5).

True infections (obligate, facultative, or opportunistic) with myxosporidian parasites in humans have not previously been recorded. Only once before have myxosporidian spores been detected in human stool samples (6). The present investigation records the detection of a second myxosporidian species in stool samples from another three patients. It is likely that further occurrences will be recorded, and diagnosticians should be aware of the possible presence of myxosporidian spores in stool samples, particularly in patients with a high dietary intake of fish. There is anecdotal evidence that myxosporidian infections are common in fish, but the actual prevalence and geographic distribution of infections in freshwater fish in Australia are unknown. Nonetheless, there is no evidence to suggest a direct association between the presence of the spores in human stools

and the occurrence of any clinical symptoms. Postmortem myoliquefaction of fish filets has been attributed to the release of proteolytic enzymes by myxosporidian cysts and spores damaged by freezing or cooking. Indeed, both mature patients had noted the unsavory nature of their recent fish meals. Nevertheless, it is not known whether the ingestion of such material would cause gastrointestinal disturbances or whether any remaining enzymes would be degraded, neutralized or simply diluted by normal gut constituents. In most instances, family members and friends of the patients in this study had also consumed fish, but they remained asymptomatic. Other enteric pathogens were also detected in two patients, *C. jejuni* in one and *B. hominis* in the other, both possibly accounting for the symptoms observed. Furthermore, the spores detected in fecal samples were intact and had not been digested in transit. Myxosporidian spores have been shown to be remarkably resistant to a range of environmental conditions and can survive passage through the alimentary tracts of piscivorous vertebrates (2). This suggests that the spores detected in these patients were fortuitous or incidental findings and were simply fish parasites which had survived passage through the gut after their ingestion with fish tissues.

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